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The effects of fish hydrolysate (CPSP) level on *Octopus maya* (Voss and Solis) diet: Digestive enzyme activity, blood metabolites, and energy balance

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Abstract

As has been demonstrated in previous studies, *Octopus maya* can be fed on artificial diets. In the present study six different diets were assayed. Five diets were designed to test the effect of percentage of inclusion of fish protein concentrate (CPSP: 0, 5, 10, 15, and 20%) and were offered to octopuses as a specifically designed artificial diet. The sixth diet consisted of frozen crab (*Callinectes* spp) and was used as control diet. Blood metabolites and energy budget of octopuses were evaluated to determine how CPSP levels modulate the digestive capacity and allow retaining energy for growth. Wild animals (316.4±9.8 g) were used in the study. Results showed that CPSP produced a positive specific growth rate (SGR, % day^{-1}) with high value in octopuses fed 15% CPSP level. A maximum growth rate of 0.86% day^{-1} was recorded in these animals, a value that is extremely low when compared with the SGR obtained when animals were fed fresh crab (3.7% day^{-1}). In general, blood metabolites were affected by diet composition, indicating that some metabolites could reflect the nutritional and/or physiological status of octopus. Preliminary reference values for *O. maya* fed crab were found for glucose (0.09± 0.02 mg/ml), lactate (0.004±0.002 mg/ml), cholesterol (0.16±0.02 mg/ml), acylglycerol (0.14±0.01 mg/ml), protein (0.37±0.04 mg/ml), hemocyanin (1.85±0.04 mmol/l), and digestive gland glycogen (1.86±0.3 mg/g). Total energy content can be used as an indicator of tissue metabolic reserves. In the present study, higher energy content in the digestive gland and muscle was observed in octopuses fed crab, followed by animals fed 15% CPSP. Results from the digestive gland indicated that the retained energy derived from glycogen, suggesting that lipids and protein were the main sources of variation linked with energy content. In general, digestive gland proteases activity and trypsin were induced in octopuses fed 15% CPSP. The capacity of *O. maya* juveniles to adjust their digestive enzymes to different types of food was evidenced. Essential amino acid content (EAA) of the diet was not a limiting factor. When dietary EAA profiles were compared with *O. maya* EAA profiles, all dietary EAA resulted in a higher concentration than whole body *octopus* composition. In the present study, all experimental groups ingested between 3300 and 4106 kJ wk^{-1} kg^{-1} without statistical differences among treatments, indicating that experimental diets were as attractive as crab. Differences were recorded in the proportion of absorbed energy (Ab, %) between CPSP-based and crab meat diets, suggesting digestion limitations associated with artificial diets. The present

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results indicate that the 15% CPSP diet had characteristics that stimulate digestive enzymes and reduce energetic costs associated with its digestion (HiE or SDA), channeling more biomass production than the other experimental diets.

Keywords: *Octopus maya*; Nutrition; Blood metabolites; Energy balance

1. Introduction

Fish protein soluble concentrates (also called CPSP) are obtained from the liquefaction of scraps of marine animals and are characterized by a high content of peptides and free amino acids as compared with the composition of native protein. Taking into account their composition, these products have been considered as an ingredient for cephalopod aquaculture diets (Le Bihan et al., 2006).

A recent study, analyzing production costs of *Octopus vulgaris* in Galicia, Spain (García-García et al., 2004), showed that the current technology for rearing octopuses is a low-profit business with a high risk mainly due to food costs. Natural prey and acquisition of wild juveniles to be used as food are expensive. For this reason, an adequate nutritional program is necessary to formulate a compounded feed for a commercially profitable production of cephalopods.

Research on artificial diets for cephalopods has been increasing in importance during the past decade. Castro (1991), using artificial diets based on shrimp paste, obtained poor survival and growth. Castro et al. (1993) used a catfish fillet-based surimi that was accepted by cuttlefish, also with poor results. A similar diet was then enriched with protein and other nutrients that promoted moderate growth (Castro and Lee, 1994). Finally, Domingues (1999) and Domingues et al. (2005) used similar diets, supplemented with protein and amino acids for cuttlefish, as well as a diet with a high protein content and adequate amino acid profile, promoting moderate growth (<0.5%BW day^{-1}). Feeding rates obtained with any of these diets (<3%BW day^{-1}) were low. Although there are studies concerning the effects of artificial diets on cephalopods (Castro, 1991; Castro et al., 1993; Castro and Lee, 1994; Domingues, 1999; Domingues et al., 2005), currently there is little information available to formulate adequate diets for these organisms.

Octopus maya, as other cephalopods, is a carnivorous species and protein is its main energy source (Van Heukelem, 1977, 1983; Segawa and Hanlon, 1988; Rosas et al., 2007). In a recent study using formulated diets we observed that dietary protein content modulates proteases activity. In that study, *O. maya* juveniles had the capacity to adjust their digestive enzymes to different types of food and protein levels. Enzyme activities appeared to be well correlated with octopus growth. General proteases and trypsin from the pancreas were well related with growth. A low activity was observed in octopuses fed 40% CP diet (negative growth rate), while a high activity was present in octopuses fed a 60% CP diet and crabs. In contrast, these same enzymes were induced in the salivary glands of octopuses fed a 40% CP diet that produced weight loss. Therefore, processes involved in digestive enzyme production in different tissues are related with the octopuses' capacity to recognize nutritional characteristics of the ingested food. The same trend has been observed with *O. vulgaris* (Best and Wells, 1983, 1984). An energetic evaluation was also made to determine how dietary protein level modulates energy channelled to biomass production. Finally, energy intake by octopuses fed 40 and 60% CP or crab meat was between 1000 and 1300 kJ kg^{-1} per week. Octopuses fed artificial diets showed a very low digestible energy (DE), indicating that diets could contain a factor, such as an anti-nutritional one, that would limit their digestibility.

Silages and protein concentrates have been used as a source of short peptides and amino acids for cephalopods. Le Bihan et al. (2006) found that cuttlefish fed *Crangon crangon* surimi soaked in silage (enriched diet) had better growth and conversion rates than cuttlefish fed a non-enriched diet. Differences among diets consisted in the supply of low molecular weight peptides and amino acids from the silage that were ingested, digested, and absorbed rapidly by growing animals.

Nutritional status is considered one of the important factors that determine the ability of animals to use the ingested nutrients. In previous studies, we determined some blood metabolites and hemocyanin, together with growth and survival in the shrimp *Litopenaeus vannamei*, *L. stylirostris*, and *L. setiferus* to assess the nutritional role of dietary carbohydrates and proteins (Rosas et al., 2000, 2001a,b, 2002). The results demonstrated that blood glucose, triacylglycerol, cholesterol, and lactate together with blood protein, hemocyanin (Hc), and osmotic pressure were good indicators of nutritional health. In cephalopods there is not enough information to correlate blood metabolites with growth and diet nor on how nutrients are mobilized. At present, knowledge on blood characteristics is scarce and focused mainly on few